Effect of *Agrobacterium* strains on gentians genetic transformation

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Introduction

Agrobacterium-mediated genetic transformation is an effective and widely used method to introduce DNA into plants. The transformation efficiency depends on many factors connected with plant species, kind of explant used, *in vitro* culture conditions and *Agrobacterium* strain.
The aim of the presented study was to determine usefulness of tested *Agrobacterium* strains for genetic modification of two species of *Gentiana* genus: *Gentiana cruciata* L. and *Gentiana tibetica* King.

Material and methods

Explants were obtained from seedlings (cotyledons, hypocotyls and roots as a whole) and young leaves (3 mm leaf fragments) of *G. cruciata* and *G. tibetica.* Explants were infected by immersion and incubation into bacterial suspension containing MS salts and vitamins, 200 µM acetosyringon and different L-glutamine concentration from 15 minutes to 120 hours (Table 1). After incubaction the explants were rinsed in water, dried and cultured on MS medium containing 30 g l^{-1} sucrose, acecosyringon and enriched (*A. tumefaciens* transformation) or not (*A. rhizogenes* transformation) with plant growth regulators (0.5 mg l^{-1} 2.4-D + 1.0 mg l^{-1} kinetin for seedlings explants, 1.0 mg l^{-1} NAA + 0.25 mg l^{-1} kinetin or 1.0 mg l^{-1} NAA + 0.5 mg l^{-1} BAP for *G. cruciata* leaf explants, 2.0 mg l^{-1} NAA + 1.0 mg l^{-1} CPPU - *G. tibetica*). 48 hours after transformation plant material was transferred onto medium with the same plant growth regulators composition and sucrose concentration with timentin or cefotaxim added to eliminate *Agrobacterium.* Two weeks later explants transformed by means of modified bacteria strains were transferred on medium containing kanamycin or BASTA (selection factor was dependant on reporter gene).

Table 1. Tested transformation conditions

L-glutamine concentrations	Incubation spans
	15’
	30’
0.0 g l^{-1}	1h
0.25 g l^{-1}	2h
0.5 g l^{-1}	4h
1.0 g l^{-1}	24h
2.0 g l^{-1}	48h
	72h
	120h

Figure 1. *G. tibetica* callus tissue on hypocotyl explant after LBA 9402 transformation

Not effective in leaf and seedlings gentians explants genetic modification. No hairy roots were regenerated. Callus tissue was formed on every of used *G. cruciata* and *G. tibetica* kind of explant (Figure 1).

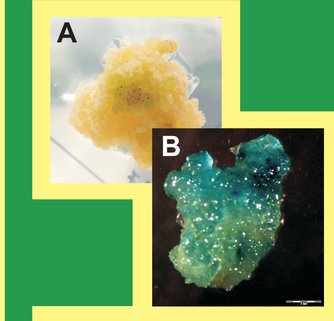


Figure 2. Callus tissue on cotyledon explant (A,B), GUS expression (B)

Figure 5. GUS-stained leaf from regenerated transformed plant

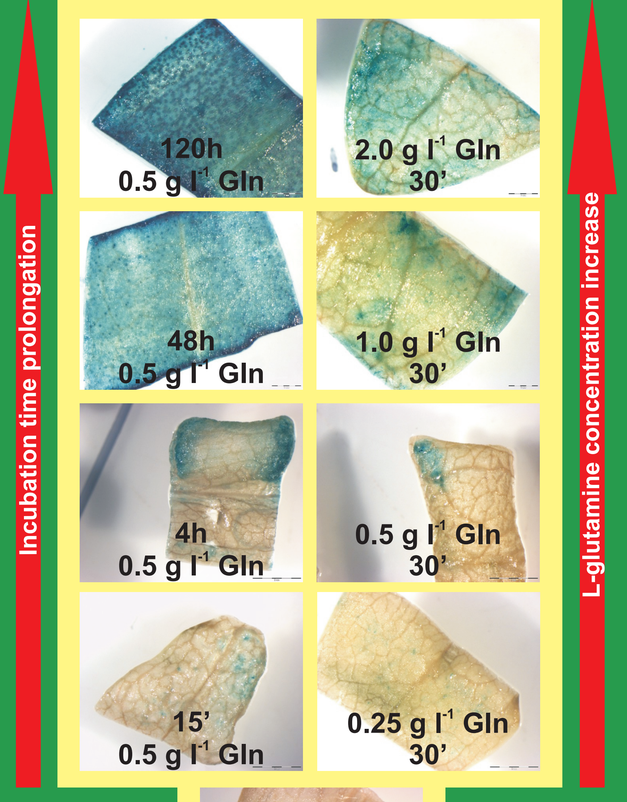
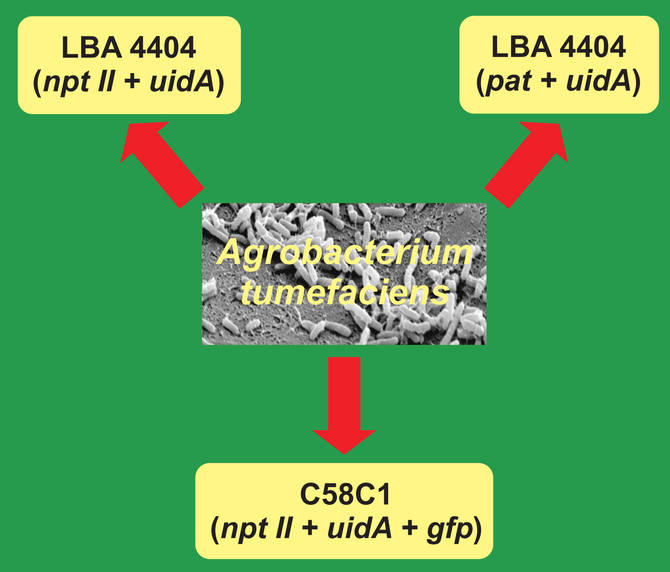


Figure 3. β-glucuroniodase activity in transformed explants after one week of culture

Not useful for gentians genetic transformation. Any results was observed. Explants were dying.

Explants did *not react* on culture conditions. No callus tissue, hairy roots or regenerated plant developed.

Strains LBA 4404 was **not useful** for seedlings and leaves transformation. Explants were dying after few weeks of culture. No transformed tissue was developed.



Gentiana tibetica

C58C1 was useful in seedling explants transformation. Callus tissue was developed on explants obtained from cotyledons. 20 % of formed callus tissue lines showed β-glucuronidase activity (Figure 2). No plants were regenerated.
C58C1 was the only strain useful for *Gentiana tibetica* leaf explants genetic modification.
Transformation efficiency (size of stained with X-gluc leaf explants area) increased with L-glutamine concentration and incubation span (Figure 3). High aminoacid content and long incubation period caused infinite bacteria cells proliferation and explants death. 2.0 g l^{-1} glutamine and 30 minutes’ incubation time was the most effective in modified callus tissue formation (Figure 4). Transformed plants were developed only in the presence of 0.25 or 0.5 g l^{-1} glutamine and one hour incubation time. β-glucuronidase activity in regenerated leaves was very low (Figure 5).

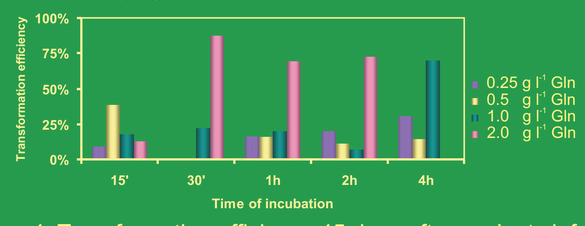


Figure 4. Transformation efficiency 45 days after explants infecion

Gentiana cruciata

Seedling explants did not react on culture conditions after *Agrobacterium* infection. Roots were formed on leaf explants. After transfer on medium containing 1.0 mg l^{-1} NAA + 0.5 mg l^{-1} BAP plants were regenerated (Figures 6-8). Repoter enzymes activity was not detected but plants grow on medium supplemented with kanamycin.

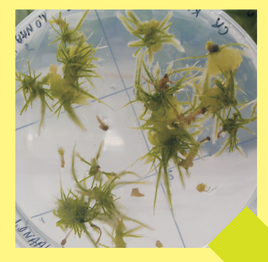


Figure 6. Formed on *G. cruciata* leaf explants roots

Figure 7. Somatic embryogenesis on roots

Figure 8. Regenerated plant

Conclusions

1. Tested *Agrobacterium rhizogenes* strains were not effectives in gentians plants genetic modifications
2. *Agrobacterium tumefaciens* was also not useful for *Gentiana cruciata* explants' transformation (β-glucuronidase activity was not observed)
3. The only strain which allowed to obtain genetic modified tissues of *Gentiana tibetica* was C58C1
4. Only cotyledons (among tested seedling explants) were useful to produce modified callus tissue but no regeneration was observed
5. 30 minutes incubation in medium with 2.0 g l^{-1} glutamine added was the most effective for *Gentiana tibetica* leaf explants transformation but genetic modified plants were regenerated in the presence of 0.25 or 0.5 g l^{-1} glutamine and one hour incubation time
6. The next stage of our study is connected with efficient plant regeneration procedure's establishment from transformed callus tissue because this phenomenon still remains not enough investigated and the regeneration efficiency is very low